Inheritance of Resistance to *Xanthomonas campestris* pv. *vesicatoria* Race P6 in *Capsicum pubescens* and Transfer of Resistance to *Capsicum annum*

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Hypothesis

The following experiment was conducted based on the hypothesis that resistance to *Xanthomonas campestris* pv. *vesicatoria* race 6 in *Capsicum pubescens* is governed by a single, dominant gene. Furthermore, it was also hypothesized that resistance from *C. pubescens* could be transferred to cultivated bell peppers *(C. annuum)* via the bridge species *C. chacoense.*

Introduction

Bacterial spot is an economically important disease of pepper throughout the world. Optimal environmental conditions for infection and spread of the bacteria are achieved mainly during rainy, warm weather. *Xanthomonas campestris* pv. *vesicatoria* overwinters in seed, soil, and plant debris. The bacteria gain entrance into the plant by way of stomata and/or wounds caused by insect, wind damage, or by mechanical damage. Dissemination of the pathogen occurs by splash dispersal, but can also result from mechanical means such as hoeing, transplanting, and harvesting (Agrios, 1997).

Bacterial spot of pepper causes damage to *Capsicum spp.* by inducing a decrease in plant growth, fruit yield, and overall fruit quality, adversely affecting marketability. Lesions may occur on leaves, fruit, stems, and petioles of infected plants. The first indication of infection is a small, yellow-green circular lesion with a yellowish halo. This lesion, with time, develops into a larger circular spot with a necrotic center region. If on foliage, the necrotic center may drop out, leaving "shot-holes" in leaves or ragged leaf edges (OSU Factsheet HYG-3123-96).

Control measures to date include (OSU Factsheet HYG-3123-96)

1. Use of pathogen-free seeds and transplants.

2. Use of sodium hypochlorite-treated seed to reduce bacterial populations.

3. Practicing crop rotation with non-host plants such as corn and soybeans so that peppers are grown in a field only once every 3 to 4 years.

4. Deep plowing to bury infected crop debris.

5. Avoiding field work when foliage is wet.

6. Eliminating wild host plants such as nightshade and ground cherry in and around field.

7. Applying copper-containing pesticides to prevent development of the pathogen.

Although these measures may help to prevent introduction of the pathogen or reduce the amount of inoculum, they are often very time consuming and/or not cost effective.

The causal agent of bacterial spot of pepper and tomato is *Xanthomonas campestris* pv. *vesicatoria* (recently proposed to be re-named *X. axonopodis* pv. *vesicatoria*). At this time, the pathogen is known to comprise three races on tomato (T1, T2, and T3) and 11 races (P0-P10) on pepper (Sahin, 1997). Race identification is determined by testing differential lines of both peppers and tomatoes and evaluating plants for a hypersensitive response (Tables 1&2)(Sahin et al., 1997). Resistant plants have a unique self-defense mechanism called the hypersensitive response. This is the ability of the plant to shut down the functioning of all cells surrounding a site of bacterial infection in the leaf tissue. By this means, a plant can stop bacteria from spreading

through plant tissue. The response is very rapid and a result can usually be obtained within 48 hours. In the test, leaves are infiltrated with a 10^8 cfu/ml bacterial suspension in an attempt to initiate the hypersensitive response. If a plant shows rapid tissue necrosis at the site of infection within 48 hours, then the plant is exhibiting a degree of resistance to that strain of the pathogen. If, however, the plant shows no tissue necrosis, then the plant is susceptible to the pathogen. By this means a quick and accurate determination is made.

Table 1. Race classification of *X. campestris* pv. *vesicatoria* strains according to the hypersensitive reaction on differential pepper lines.

	Pepper differential lines			
Race	ECW	ECW-10R	ECW-20R	ECW-30R
P0	C[1]	HR[2]	HR	HR
P1	C	C	HR	HR
P2	C	HR	HR	C
P3	C	C	HR	C
P4	C	C	C	HR
P5	C	HR	C	C
P6	C	C	C	C

[1]C = compatible (susceptible) reaction
[2]HR = hypersensitive reaction (incompatible reaction)

Table 2. Race classification of *X. campestris* pv. *vesicatoria* strains according to the hypersensitive reaction on differential tomato lines.

	Tomato differential lines		
Race	OH 8245	H 7998	PI 128216
T1	C[1]	HR[2]	C
T2	C	C	C
T3	C	C	HR

[1]C = Compatible reaction
[2]HR = Hypersensitive reaction (incompatible reaction)

Many commercial bell pepper cultivars are resistant to one or more (Table 3) races of *X. campestris* pv. *vesicatoria.* However, none are resistant to race P6 which was first reported in Ohio in 1994.(Sahin and Miller 1997). Host resistance is governed by 3 genes (Bs1, Bs2, and Bs3)(Table 3). Sahin and Miller identified a fourth source of resistance to *X. campestris* pv. *vesicatoria* P6 in *Capsicum pubescens* PI 235047, proposed to be designated Bs4 (Sahin and Miller 1997). The Bs1, Bs2, and Bs3 resistance genes are simply inherited and segregate independently of each other. The inheritance of the proposed Bs4 gene is unknown. In order for a resistant reaction to occur, an avirulence gene must be present in the pathogen along with a resistance gene in the host (Minsavage et al. 1989).

Table 3. Proposed race classification of *X. campestris* pv. *vesicatoria* pepper strains based on hypersensitive reaction (HR) on pepper differential lines and *Capsicum pubescens* PI 235047.

	Pepper differential lines				
Races	ECW	ECW-10R *Bs 1*	ECW-20R *Bs 2*	ECW-30R *Bs 3*	PI 235047 *Bs 4*[1]
P0	-[2]	HR	HR	HR	HR
P1	-	-	HR	HR	HR
P2	-	HR	HR	-	-
P3	-	-	HR	-	HR
P4	-	-	-	HR	HR
P5	-	HR	-	-	-
P6	-	-	-	-	HR
P7	-	-	HR	HR	-
P8	-	-	HR	-	-

[1]Proposed designation.
[2]Compatible interaction.

It would be advantageous to transfer, by plant breeding, the gene or genes responsible for resistance to *X. campestris* pv. *vesicatoria* P6 in *C. pubescens* PI 235047 into cultivated peppers. However, the desired cross between *C. pubescens* and *C. annuum* is not directly possible due to breeding incompatibilities of these two species, specifically barriers in the ovary and on the stigma (Zijlstra et al., 1991). In order to successfully attempt to transfer resistance to *X. campestris* pv. *vesicatoria*, an alternative to direct crossing must be utilized. The species *C. chacoense* was proposed as a bridge between the other two species. *C. chacoense* was chosen due to the fact that it has been documented in other studies (Zijlstra et al., 1991) that pollen tubes from *C. pubescens* as the male parent can penetrate the egg cells of *C. chacoense* as the female parent, and

likewise, the pollen tubes from *C. chacoense* as the male parent can penetrate the egg cells of *C. annuum* as the female parent. Although pollination has been documented as successful, it is not known whether embryos from this cross can develop into mature offspring. It has been determined that *C. pubescens* crosses readily with *C. cardenasii* and *C. eximium* (Pickersgill, 1997).

If viable offspring were obtained, it would be necessary to distinguish between hybrid offspring and those offspring resulting from self-pollination. Preliminary indications of parentage could be made by examining morphological characters of the progeny in comparison to the parents. There where a few very distinct differences between the various species of parent plants and also small differences among the different cultivars of each species. These phenotypic differences of size, shape, and color can be used to evaluate whether or not the offspring are hybrids. Progeny exhibiting all phenotypic traits from a single parent species are likely to be the result of a self-pollination, while those progeny with a few phenological traits from two different parents are assumed to be hybrids.

An additional means of determining the parentage of any offspring is by a form of genetic testing called RAPD (Random Amplified Polymorphic DNA analysis)(Newbury and Ford-Lloyd. 1992). The testing consists of DNA extraction from pepper leaves, Polymerase Chain Reaction (PCR) to amplify DNA (including denaturing of template DNA, annealing of primers, and extension of DNA), and visualization of DNA fragments by size in agar gel electrophoresis (Figure 1). The DNA banding patterns (fingerprints) of the parent species are compared to those of the offspring. If the parent DNA fingerprint

is the same as that of the progeny, the progeny are the result of self-fertilization. If, however, the progeny show a DNA fingerprint unlike either parent, they are hybrids and the cross was a success. Once all of the hybrids have been selected, they are then tested for resistance to the *X. campestris* pv. *vesicatoria* race 6.

Assuming that resistance to *X. campestris* pv. *vesicatoria* race P6 is governed by a single gene, as in the model in Figure 2, all F1 progeny will be heterozygous resistant. The F2 progeny, then, will show a 3:1 ratio of resistance to susceptibility. If, however, resistance is governed by multiple genes, as in the model in Figure 3, all F1 progeny can be expected to be heterozygous resistant, while the F2 progeny exhibit a 9:7 ratio of resistance to susceptibility.

Figure 2

Single Gene Inheritance

		R[1]	R	
Generation	r[2]	Rr	Rr	= 100% resistant
1	r	Rr	Rr	

		R	R	
Generation	R	RR	Rr	= 3 resistant : 1 susceptible
2	r	Rr	rr	

[1] R = Dominant gene for resistance.
[2] r = Recessive gene for susceptibility

Figure 3

Multiple Gene Inheritance

		R^1T^2	RT
Generation	r^3t^4	RrTt	RrTt
1	rt	RrTt	RrTt

= 100% resistant

Generation 2	RT	rT	Rt	rt
RT	RRTT	RrTT	RRTt	RrTt
rT	RrTT	rrTT	RrTt	rrTt
Rt	RRTt	RrTt	RRtt	Rrtt
rt	RrTt	rrTt	Rrtt	rrtt

= 9 resistant : 7 susceptible

[1]R = Dominant gene for resistance.
[2]r = Recessive gene for resistance.
[3]T = Dominant gene for resistance.
[4]t = Recessive gene for resistance.

Material & Methods

Bacterial Strains Used. The strains of *X. campestris* pv. *vesicatoria* used in this study are shown in Table 4

Table 4. Source of strains of races *X. campestris* pv. *vesicatoria* used in this study.

Strain	Race	Source
V19	T1P0	Pepper
118	T1P1	Pepper, Seneca County, OH
89	T1P2	Pepper
108	T1P3	Pepper, Wayne County, OH
181	T1P4	
206	T1P5	Tomato, Wood County, OH
17b	T1P6	Pepper
117	T1P7	Pepper, Wood County, OH
110c	T1P8	Pepper, Wayne County, OH
420	T1P10	Pepper, NC, D. Ritchie, NCSU

***Capsicum spp.* tested.** The lines of *Capsicum spp.* tested in this study are shown in Table 5.

Table 5. *Capsicum spp.* plant introduction lines and varieties tested for resistance to *X. campestris* pv. *vesicatoria* race P6.

PI line	No. of Plants	Species
235047	8	*C. pubescens*
355394	4	*C. pubescens*
355811	2	*C. pubescens*
387838	1	*C. pubescens*
497676	2	*C. pubescens*
585259	2	*C. pubescens*
585262	3	*C. pubescens*
585264	2	*C. pubescens*
585265	3	*C. pubescens*
585266	10	*C. pubescens*
585268	2	*C. pubescens*
585267	5	*C. pubescens*
585270	2	*C. pubescens*
585271	4	*C. pubescens*
585273	3	*C. pubescens*
585274	4	*C. pubescens*
585276	3	*C. pubescens*
590503	1	*C. pubescens*
593621	3	*C. pubescens*
593633	2	*C. pubescens*
593644	3	*C. pubescens*
594141	2	*C. eximium*
590507	1	*C. cardenasii*
Early Calwonder(ECW)	5	*C. annuum*
ECW 10R	5	*C. annuum*
ECW 20R	5	*C. annuum*
ECW 30R	5	*C. annuum*

Hypersensitive Response Tests. Plants of *X. campestris* pv. *vesicatoria* race P6 resistant PI line 235047, *X. campestris* pv. *vesicatoria* P6 susceptible PI line 585266, and the *C. annuum X. campestris* pv. *vesicatoria* race differentials (Early Calwonder(ECW), ECW 10R, ECW 20R, and ECW 30R) were tested for the hypersensitive response to *X. campestris* pv. *vesicatoria* races T1P0, T1P1, T1P2, T1P3, T1P4, T1P5, T1P6, T1P7, T1P8, and T1P10. Also, hypersensitive response tests were administered to each of the *Capsicum spp.* of interest (Table 5) to determine their resistance or susceptibility to *X. campestris* pv. *vesicatoria* race P6. Each of the two hypersensitive response tests were done twice.

Protocol for the Hypersensitive Response Test

1) Remove bacterial strain from -80 C freezer where it is stored in a 15% glycerol solution.

2) Streak culture onto Yeast Dextrose Carbonate Agar (YDC) medium and incubate at 28 C for 48 hours.

3) Wash bacteria from plate with a sterile glass spreader and suspend in sterile distilled water.

4) Adjust bacterial concentration to 10^8 cfu/ml (absorbance at 600 nm of 0.1).

5) Infiltrate bacterial solution into the intercostal area of the leaves using a 3 cc syringe without the needle.

6) Infiltrate control plants with sterile water; also use the known resistant ECW lines as controls.

7) Incubate plants on a bench in a greenhouse organized randomly for 24-48 hours at 20-28 C.

***Capsicum spp.* Crosses.** All *Capsicum pubescens* PI lines shown to be resistant to *X. campestris* pv. *vesicatoria* were crossed reciprocally with the known homozygous susceptible line, PI 585266. Seeds were obtained from these crosses when fruit reached maturity (approximately two months) and were then planted to produce F1 progeny, which were to be tested for HR.

Female flowers were emasculated using forceps, being careful not to harm the stigma. Pollen was then collected from the male parent plant using the blade of a scalpel, blackened by flaming to enhance pollen visibility, by scraping the anthers. Collected pollen was then smeared onto the stigma using the scalpel.

DNA Extraction. DNA extraction was performed using the method described below.

C-TAB Extraction from Aldrich "Vega Method"

1) CTAB Buffer

1.4 M NaCl	8.2 g
100 mM Tris-HCL, pH 8.0	1.2 g
20 mM EDTA	0.7 g
2% CTAB	2.0 g
	100 ml

This might not go into solution unless autoclaved. Add 80 µl 10 mM B-mercaptoethanol when cool.

2) Weigh out 0.2 g fresh pepper leaf tissue. Then grind with mortar and pestle using liquid nitrogen, and put sample in a 1.5 ml microfuge tube.

3) Add 600 µl CTAB buffer.

4) Cap tube and incubate at 65 C for 15 minutes. This step releases the DNA from the cells.

5) Cool to room temperature and add an equal amount of 24:1 chloroform: iso-amyl alcohol.

6) Vortex until white. Repeat step 5 if necessary to obtain white solution.

7) Centrifuge at 12,000 rpm for 7 minutes to separate phases.

8) Save the aqueous layer, leaving the interface behind.

9) Add 60 µl 3 M NaAcetate, pH 5.2 and 600 µl isopropanol.

10) Let stand at room temperature for 30 minutes and centrifuge at 12,000 rpm for 15 minutes.

11) Remove alcohol, air dry and resuspend in 30 µl TE buffer.

12) Store in -20 C or -80 C freezer.

13) Make a 1:100 dilution by placing 190 µl TE buffer in three new tubes, followed by 10 µl resuspended DNA. Also, make one blank by placing 200µl TE buffer in a fourth tube.

14) Determine concentration of each solution using the spectrophotometer (260/280nm ratio).

Preparation of DNA Samples.

Spectrophotometer Results

>WAVELENGTH SCAN TABLE< Date: 7 / 13 / 98

Function: Absorbance

Wavelength: 260-280

Sample Number Sample ID	Wavelength 260	Wavelength 280	260/280	Working [DNA] ng/µl	Stock [DNA]
(1) **Blank**	0.0000	0.0000	-------	---------	-------
(2) ***C. annuum***	0.43054	0.26016	1.65	21.53	2153
(3) ***C. chacoense***	0.14227	0.09941	1.43	7.11	711
(4) ***C. pubescens***	0.74246	0.39236	1.89	37.12	3712

Calculations

DNA [Working] = A260 x 50 = ng/µl

DNA [Stock] = A260 x 50 x dilution factor (100) = ng/µl

Adjusted Concentration of Working DNA to 2 ng/μl	**Total**
1) *C. annuum* - 21.53 ng/μl = 19 μl DNA + 181 μl TE	200 μl volume
2) *C. chacoense* - 7.11 ng/μl = 56 μl DNA + 144 μl TE	200 μl volume
3) *C. pubescens* - 37.12 ng/μl = 11 μl DNA + 189 μl TE	200 μl volume

RAPD Analysis. RAPD analysis was executed on all three parent species of pepper. Seven microfuge tubes were labeled for each primer used (primers 244, 262, OPH-1, OPH-2, OHP-3, OPH-4, OPH-5, OPH-6, OPH-7, OPH-8, OPH-9, OPH-10, OPH-13, OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-9, and OPA-13). The tubes were labeled with the species abbreviations Ca, Cc, and Cp for *Capsicum annuum, Capsicum chacoense, and Capsicum pubescens* respectively. Each species had two repetitions and therefore one of each pair of tubes was labeled with a subscript numeral 2. Each tube was also labeled with the primer number used for the set. The protocol is shown below.

RAPD Testing Protocol

Master mix per tube			**Total**
destilled H_2O	10.80 µl x 8	=	86.40 µl
$MgCl_2$	1.5 µl x 8	=	12.0 µl
10 X buffer	2.5 µl x 8	=	20.0 µl
dNTPs mix	2.5 µl x 8	=	20.0 µl
Primer	2.5 µl x 8	=	20.0 µl
Taq Polymerase	0.2 µl x 8	=	1.6 µl
	20.0 µl		160.0 µl

1) Mix the contents of the master mix very well, briefly and gently.

2) Dispense 17.5 µl of master mix into each labeled microfuge tube.

3) Add 5.0 µl of template DNA (~10 ng) to all tubes except one negative control.

4) Add 5.0 µl dH_2O to the negative control.

5) Add one drop of mineral oil to each tube.

The PCR Reaction. Samples were then placed into a Thermolyne thermocycler. The two different programs used in this study are shown below. Program number 89 is the result of two programs, 50 and 51, which are linked. Periods of 94 C temperature served to denature the template DNA, 30 C served to anneal primers, and 72 C served to extend

primers. Program number 22 is the result of two programs, 10 and 11, which are linked. In this program, a 94 C => 40 C => 72 C temperature change was used respectively. The exact programs are shown below.

Program # 50

	Degrees C	Seconds
Step 1:	94	0
Step 2:	94	30
Step 3:	30	0
Step 4:	30	60
Step 5:	72	0
Step 6:	72	120 (2 minutes)

Total of 44 cycles

Program # 51

	Degrees C	Seconds
Step 1:	94	0
Step 2:	94	30
Step 3:	30	0
Step 4:	30	60
Step 5:	72	0
Step 6:	72	600 (10 minutes)

Total of 1 cycle

Post Dwell at 4 C for 24 hours.

Program #22 (10 & 11 are linked)

Program #10

	Degrees	Seconds
Step 1:	94	0
Step 2:	94	60
Step 3:	40	0
Step 4:	40	60
Step 5:	72	0
Step 6:	72	120 (2 minutes)

Total of 44 cycles.

Program #11

	Degrees C	Seconds
Step 1:	94	0
Step 2:	94	60
Step 3:	40	0
Step 4:	40	60
Step 5:	72	0
Step 6:	72	600 (10 minutes)

Total of 1 cycle.

Post Dwell at 4 C for 24 hours.

Agarose Gel Electrophoresis. After PCR is complete, samples are loaded into an agarose gel for gel electrophoresis. The protocol for agarose gel electrophoresis is shown below.

Protocol for Agarose Gel Electrophoresis

1) Add together in a flask 1.4 g agarose and 100 ml 1 X TBE buffer.

2) Microwave until all the agarose has dissolved.

3) Cool the flask in a water bath to about 50-70 C, gently shaking, but without creating bubbles, to assure even cooling.

4) Add to flask, 10 μl of 1 mg/ ml Ethidium bromide.

5) Clean the electrophoresis tray with water and dry.

6) Seal edges of tray with rubber stoppers.

7) Pour the gel into the tray and put the comb into its position.

8) After the gel has completely hardened (this takes ~ 20-30 minutes), carefully remove the comb and stoppers.

9) Mount the gel carefully in the electrophoresis tank.

10) Make sure that the gel is covered with about 2-3 mm 1 X TBE buffer.

11) Take a 10 μl sample from underneath the mineral oil and mix it with 1.5 μl tracking dye on a piece of parafilm. Load gel, being careful not to destroy the bottom of the wells. Reduce the movements of your hands as much as possible in order not to loose the sample.

12) Close the lid and attach the electric wires so that the DNA will migrate to the anode (red). Run the gel for 1-1.5 hours at 90 volts.

13) After 1-1.5 hours, disconnect the power and remove the lid.

→ Be very careful to wear gloves when removing the gel and processing photo images. Ethidium bromide is a very powerful mutagen.

Results. All of the *Capsicum spp.* used in this study were noticeably different from each other in appearance. The *C. pubescens* line, PI 235047, was a densely hairy, larger leafed variety showing little purpling in the leaves and stems. It also had relatively large purple flowers, which exhibited white highlights and formed large green fruit that showed little purpling. Fruit of this line were yellow at maturity. Another plant introduction line of *C. pubescens* used in this experiment, PI 585266, also had leaves that were large and hairy but smaller than those of PI 235047. Foliage was slightly darker in color than PI 235047 and stems and leaves showed a slight purpling. Flowers were also relatively large, but showed no white highlight and formed green fruit of the same size as PI 235047 that showed a slight purpling in pigment and turned red at maturity. Two lines of *C. chacoense* were also used, PI 260431 and PI 237429. PI 260431 had much smaller, lighter green foliage than *C. pubescens* and was not hairy. Flowers were also very small and were white with yellow anthers. Fruit were green in color but were only a fraction of the size of those of *C. pubescens* and were more elongate in shape. PI 237429, on the other hand, was of the same general size and shape as PI 260431, but the flowers contained anthers of a darker purplish

color borne in a white flower. The bell pepper (*C. annuum)* cultivar Early Calwonder (ECW), used in this study was of the same general size and shape as *C. pubescens* (PI235047), although the leaves were absent of hair and lighter in color, and the fruit were much larger.

Hypersensitive Response Tests. The results of the two hypersensitive response tests performed in this study are shown in Tables 6 and 7.

Table 6. Response of PI 235047 and PI 585266 to *X. campestris* pv. *vesicatoria* races P0, 1, 2, 3, 4, 5, 6, 7, 8, and 10.

PI line	ID	P0	P1	P2	P3	P4	P5	P6	P7	P8	P10
235047	.1	+[1]	+	-[2]	+	+	+	+	-	+	+
	.2	+	+	-	+	+	+	+	-	+	+
	.3	+	+	-	+	+	+	+	-	+	+
	.4	+	+	-	+	+	+	+	-	+	+
	.5	+	+	-	+	+	+	+	-	+	+
	.6	+	+	-	+	+	+	+	-	+	+
	.7	+	+	-	+	+	+	+	-	+	+
	.8	+	+	-	+	+	+	+	-	+	+
	.9	+	+	-	+	+	+	+	-	+	+
	.10	+	+	-	+	+	+	+	-	+	+
	.11	+	+	-	+	+	+	+	-	+	+
	.12	+	+	-	+	+	+	-	-	+	+
	.13	+	+	-	+	+	+	-	-	+	+
	.14	+	+	-	+	+	+	+	-	+	+
	.15	+	+	-	+	+	+	+	-	+	+
	.16	+	+	-	+	+	+	+	-	+	+
	.17	+	+	-	+	+	+	-	-	+	ND[3]
	.18	+	+	-	+	+	+	+	-	+	+
	.19	+	+	-	+	+	+	+	-	+	+
	.20	-	+	-	+	+	+	+	-	+	+
	.21	+	+	-	+	+	+	+	-	+	+
	.22	+	+	-	+	+	+	+	-	ND	+
	.23	+	+	-	+	+	+	+	-	+	+
	.24	-	+	-	+	+	+	+	-	+	+
	.25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	.26	+	+	-	ND	+	+	+	-	+	+
	.27	-	-	-	+	+	+	+	-	+	+
	.28	+	-	-	+	+	+	+	-	+	+
	.29	+	+	-	+	ND	-	+	-	+	+
	.30	+	+	-	+	+	+	+	-	+	+
	.31	-	+	-	+	+	+	+	-	+	+
	.32	+	+	-	+	+	+	+	-	+	+
	.33	+	+	-	+	+	+	+	-	+	+
	.34	+	+	-	+	+	+	+	-	+	+
	.35	-	+	-	+	+	+	+	-	+	+
	.36	+	+	-	+	+	+	+	-	+	+
	.37	+	+	-	+	+	+	+	-	+	+
	.38	+	+	-	+	+	+	+	-	+	+

Table 6 continued. Response of PI 235047 and PI 585266 to *X. campestris* pv. *vesicatoria* racesP0, 1, 2, 3, 4, 5, 6, 7, 8, and 10.

PI line	ID	P0	P1	P2	P3	P4	P5	P6	P7	P8	P10
585266	.1	-	-	+	+	-	+	-	-	-	-
	.2	-	-	+	+	-	+	-	-	-	-
	.3	-	-	+	-	+	+	-	-	-	-
	.4	-	-	+	+	-	+	-	-	-	-
	.5	-	-	+	-	-	+	-	-	-	-
	.6	-	-	+	-	-	+	-	-	-	-
	.7	-	-	+	-	-	+	-	-	-	-
	.8	-	-	+	-	-	+	-	-	-	-
	.9	-	-	+	-	-	+	-	-	-	-
	.10	-	-	+	-	-	+	-	-	-	-
	.11	-	-	+	-	-	+	-	-	-	-
	.12	-	-	+	-	-	+	-	-	-	-
	.13	-	-	+	-	-	+	-	-	-	-
	.14	-	-	+	-	-	+	-	-	-	-
	.15	-	-	+	-	-	+	-	-	-	-
	.16	-	+	+	-	-	+	-	-	-	-
	.17	-	-	+	-	-	+	-	-	-	-
	.18	-	+	+	-	-	+	-	-	-	-
	.19	-	-	+	-	-	+	-	-	-	-
	.20	-	-	-	-	-	+	-	-	-	-
	.21	-	-	-	-	-	+	-	-	-	-
	.22	-	+	+	-	-	+	-	-	-	-
	.23	-	+	+	-	-	+	-	-	-	-
	.24	-	+	+	-	-	+	-	-	-	-
ECW	.1	-	-	-	-	-	-	-	-	-	-
	.2	-	-	-	-	-	-	-	-	-	-
	.3	-	-	-	-	-	-	-	-	-	-
	.4	-	-	-	-	-	-	-	-	-	-
	.5	-	-	-	-	-	-	-	-	-	-
	.6	-	-	-	-	-	-	-	-	-	-
	.7	-	-	-	-	-	-	-	-	-	-
ECW 10R	.1	+	-	+	-	-	-	-	-	-	-
	.2	+	-	+	-	-	-	-	-	-	-
	.3	+	-	+	-	-	-	-	-	-	-
	.4	+	-	+	-	-	-	-	-	-	-
	.5	-	-	+	-	-	-	-	-	-	-
	.6	+	-	+	-	-	-	-	-	-	-
	.7	+	-	+	-	-	-	-	-	-	-

Table 6 continued. Response of PI 235047 and PI 585266 to *X. campestris* pv. *vesicatoria* racesP0, 1, 2, 3, 4, 5, 6, 7, 8, and 10.

ECW 20R	.1	-	+	+	+	-	-	-	-	-	-
	.2	-	-	-	-	-	-	-	-	-	-
	.3	-	-	-	-	-	-	-	-	-	-
	.4	+	-	+	-	-	-	-	-	-	-
	.5	-	-	-	+	-	-	-	-	-	-
	.6	-	-	-	-	-	-	-	-	-	-
	.7	-	-	+	-	-	-	-	-	-	-
ECW 30R	.1	+	+	-	-	+	-	-	+	-	-
	.2	+	+	-	-	+	-	-	+	-	-
	.3	+	+	-	-	+	-	-	+	-	-
	.4	+	+	-	-	+	-	-	+	-	-
	.5	+	+	-	-	-	-	-	+	-	-
	.6	+	+	-	-	+	+	-	+	-	-
	.7	+	+	-	-	+	-	-	+	-	-

[1] + denotes a hypersensitive response.

[2] - denotes no hypersensitive response.

[3]ND denotes no data.

*There are small deviations in the table due to experimental error.

Table 7. Response of *Capsicum spp.* to *X. campestris* pv. *vesicatoria* race P6.

PI line	ID	Lf 1[1]	Lf 2	Lf 1	Lf 2
235047	A	+[2]	+	+	+
	B	+	+	+	+
	C	+	+	+	+
	D	+	+	+	+
	E	+	+	+	+
	F	+	+	+	+
	G	+	+	+	+
	H	+	+	+	+
355394	A	+	+	+	+
	B	+	+	+	+
	C	+	+	+	+
	D	+	+	+	+
355811	A	-[3]	-	-	-
	B	-	-	-	-
387838	A	-	-	-	-
497676	A	-	-	-	-
	B	-	-	-	-
585259	A	-	-	-	-
	B	-	-	-	-
585262	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
585264	A	-	-	-	-
	B	+	+	+	+
585265	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
585266	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
	D	-	-	-	-
	E	-	-	-	-
	F	-	-	-	-
	G	-	-	-	-
	H	-	-	-	-
	I	-	-	-	-
	J	-	-	-	-
585268	A	-	-	-	-
	B	-	-	-	-

Table 7 continued. Response of *Capsicum spp.* to *X. campestris* pv. *vesicatoria* race P6.

PI line	ID	Lf 1[1]	Lf 2	Lf 1	Lf 2
585269	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
	D	-	-	-	-
	E	-	-	-	-
585270	A	-	-	-	-
	B	-	-	-	-
585271	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
	D	-	-	-	-
585273	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
585274	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
	D	-	-	-	-
585276	A	-	-	-	-
	B	-	-	-	-
	C	-	-	-	-
590503	A	-	-	-	-
593621	A	+	+	+	+
	B	+	+	+	+
	C	+	+	+	+
593633	A	+	+	+	+
	B	+	+	+	+
593644	A	+	+	+	+
	B	+	+	+	+
	C	+	+	+	+
594141	A	-	-	-	-
	B	-	-	-	-
590507	A	-	-	-	-

In each of the two repetitions of this test, two leaves on the plant were infiltrated with *X. campestris* pv. *vesicatoria* race 6. Pepper lines EC 10R, EC 20R, and EC 30R, all known
homozygous resistant varieties were used for the control

[1] Lf 1 and Lf 2 stands for leaf one and leaf two respectively.

[2] + Denotes hypersensitive response.

3 - Denotes no hypersensitive response.

Successful Crosses. The known resistant line PI 235047 and the known susceptible line PI 585266 have been successfully crossed and thus, their progeny can be used for determination of the of the inheritance of the proposed Bs4 resistance gene (Table 8). Although these crosses have been made, there exists only three crosses for which reciprocals have been successfully made. The existence of reciprocals is quite important to determining the inheritance of a gene and thus more attempts must be made to cross the remaining lines reciprocally.

The final two crosses, PI 355394A x 585266F and 585266A x 593621C, are the only successful crosses that have come from the newly identified resistant PI lines (Table 6). More crosses to the other newly identified resistant PI lines must be made along with their reciprocals in order to determine the inheritance of these genes in the future.

Table 8. Successful crosses made between resistant and susceptible *Capsicum pubescens* PI lines 585266 (susceptible), 235047 (resistant), and 355394 (resistant).

Cross (female x male)	No. of Crosses Made
585266A[1] x 235047H	1
585266B x 235047H	2
585266C x 235047H	1
585266D x 235047H	2
585266F x 235047H	2
585266H x 235047H	4
235047H x 585266C	4
235047H x 585266D	2
355394A x 585266F	1
585621C x 593621C	1

[1]A, B, C, D, F, and H depict which individual plant of that PI line was used for that specific cross.

Discussion. In the course of this experiment many confounding variables arose. First and foremost, two weeks into the experiment it was observed that crossed buds were beginning to abort and fall off. By the beginning of the fourth week, all previously made crosses had aborted as had many flowers. There are several reasons why the pepper plants would abort their flowers. The greenhouse in which the plants were kept was found to have an infestation of insect pests called Thrips, which placed stress on the plants. It was also found that temperatures above 90C can cause sterility in pepper pollen, and therefore the female plants may not have been adequately pollinated. Furthermore, in other studies, it has been found that high humidity can also hamper pollination (Joseph Jacobs, pers. comm.). Finally, the general stress of petal and anther removal may have added to stress already on the plants.

Another confounding variable arose in the location of a representative strain of each race of *X.campestris* pv *vesicatoria* desired. Only ten of the eleven desired strains were located. A representative strain of T1P9 was unaccounted for. This strain must be obtained from other institutions in the future.

In the course of this experiment it has also been found that the time period allotted by this program is not adequate for agricultural research of this nature. Growing plants to reproductive maturity is a process that requires more time, especially if more than one generation of plants is to be grown. Also, DNA replication is a very sensitive process and it takes much practice to learn the techniques necessary to perform it. Therefore, this experiment will continue after this program is completed.

Finally, our limited success with *C. chacoense* as a bridge species made it impossible to conduct the rest of the tests because no viable offspring were obtained. These tests, however, have already been studied and will be very useful in future research.

Avenues for further research. The most probable avenue for further research is the selection of an alternate bridge species. Seeds from the species *Capsicum eximium* and *Capsicum cardenasii* have already been obtained to test as alternate bridge species in the transfer of *X. campestris* pv.*vesicatoria* race 6 resistance genes.

An equally interesting avenue for further research is testing for genetic variance among the different races of the pathogen. This may serve to find an easier method of strain identification, or may serve to find general information about the pathogen itself.

The process of embryo rescue may also be used in the future if crosses will not mature successfully as in the case of the *C. chacoense* X *C. pubescens* and *C. annuum* X *C. chacoense* crosses.

The results desired from the *X. campestris* pv. *vesicatoria* race 6 portion of this experiment have not yet been attained. Seed from crosses between PI 585266, the known homozygous resistant, and the other resistant lines of unknown genetics has been collected, and in some cases are already growing. However, these seedlings must yet be grown, tested using the HR test, and then selfed seed must be collected from these progeny as well. When this second generation is planted, grown, and HR tested, only then will we know whether the original unknown parent was homozygous dominant or heterozygous.

The results obtained from the HR tests using *X campestris* pv. *vesicatoria* races T1P0, T1P1, T1P2, T1P3, T1P4, T1P5, T1P6, T1P7, T1P8, and T1P10 were consistent and reproducible (Table 6). They indicate that PI 235047 bears the most effective resistance to multiple races of *X. campestris* pv. *vesicatoria*. It is resistant to the T1P0, T1P1, T1P3, T1P4, T1P5, T1P6, T1P8, and T1P10 races and susceptible to only T1P2 and T1P7. The ECW line is susceptible to all races of *X.campestris* pv. *vesicatoria* tested. The ECW 10R line is resistant to T1P0 and T1P2 and is susceptible to T1P1, T1P3, T1P4, T1P5, T1P6, T1P7, T1P8, and T1P10. The ECW 20R line is susceptible to all races of *X.campestris* pv. *vesicatoria* tested. The ECW 30R line is resistant to T1P0, T1P1, T1P4, and T1P7 while it is susceptible to T1P2, T1P3, T1P5, T1P6, T1P8, and T1P10. PI 585266 is resistant to T1P2 and T1P5, while it is susceptible to T1P0, T1P1, T1P3, T1P4, T1P6, T1P7, T1P8, and T1P10.

Knowing what we now do regarding the resistance of PI 585266 to the 10 different races of *X. campestris* pv. *vesicatoria* tested, namely that it is resistant to T1P2 and T1P5, we can now test the offspring of this cross for similar traits. There is a possibility that offspring may have been generated that possess resistance alleles to *X. campestris* pv. *vesicatoria* races T1P2, T1P5, and T1P6. One avenue for further research is the testing of all of the unknown resistant lines for an HR using *X. campestris* pv. *vesicatoria* races T1P0, T1P1, T1P2, T1P3, T1P4, T1P5, T1P7, T1P8, and T1P10 in addition to race T1P6 that was used for genetic testing. It would be very interesting to determine the reaction of these plants to the races of the pathogen, besides T1P6.

The mentors to this project played a crucial role in its founding hypothesis and also the execution of all portions of the experiment. Dr. Sally A. Miller, associate

professor of Plant Pathology at The Ohio State University, proposed the project and gave guidance as to the specific trials it should include and also a protocol for these trials. Dr. David Francis, a research scientist in the Department of Horticulture and Crop Science at The Ohio State University, was very supportive in giving a protocol and demonstration in cross breeding and also played a major role in finding solutions to problems in the area of cross breeding as well.

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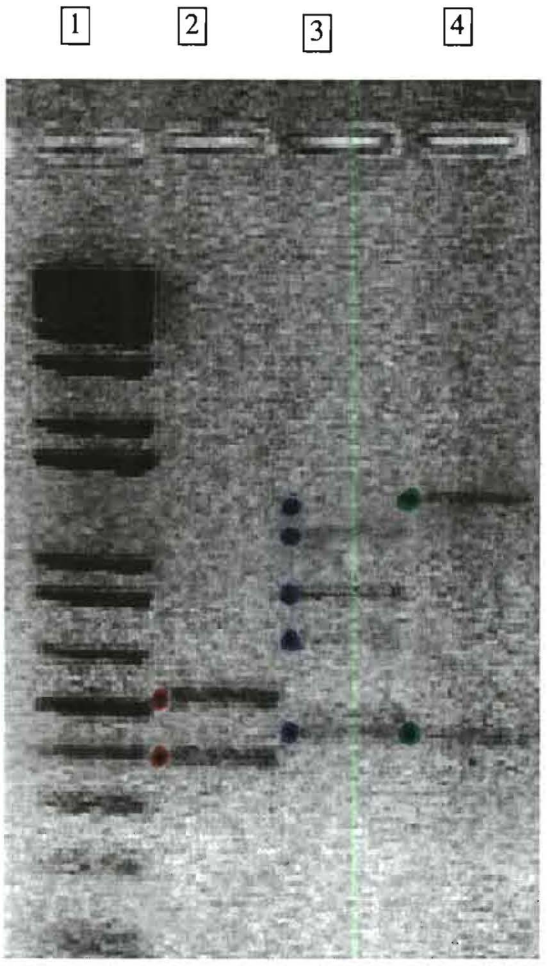
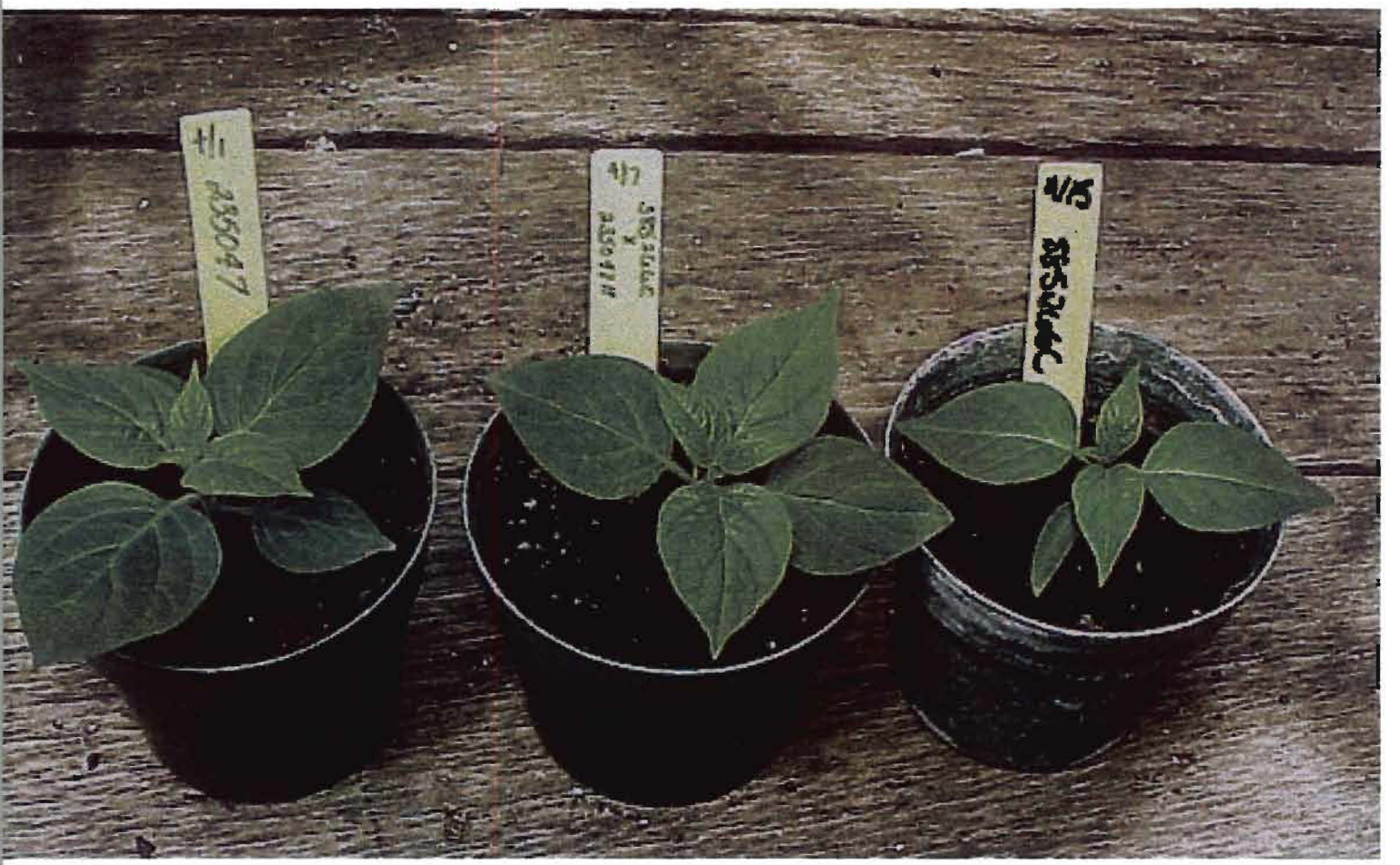


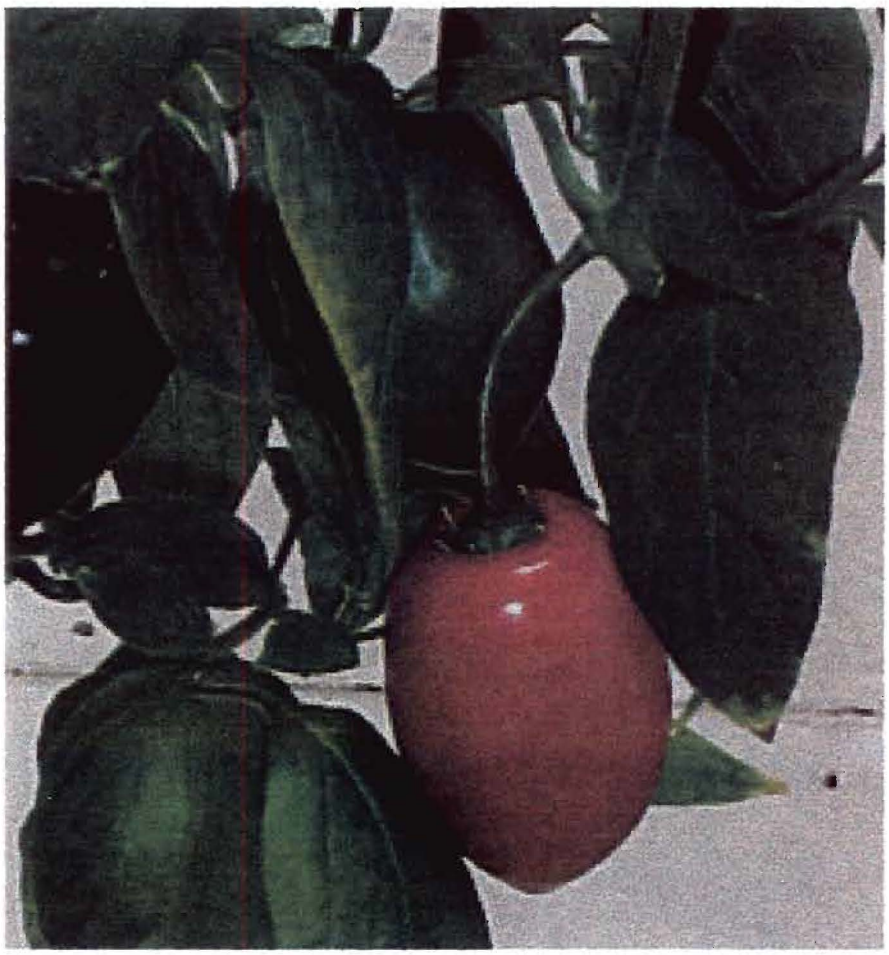
1.) DNA Ladder

2.) C. pubescens

3.) C. annuum

4.) C. chacoense


4/1
4/7
4/15




585266A
X
593621C